REMARKS/ARGUMENTS

Status of Claims

Claims 26, 27, and 30 have been amended.

Claims 3, 5, 23-25, 28, 29, 31-34, 39-43, and 46-52 have been canceled.

Thus, claims 1, 2, 4, 6-22, 26, 27, 30, 35-38, 44-45, and 53-56 are currently pending in this application.

Applicants hereby request further examination and reconsideration of the presently claimed application.

Claim Amendments

Applicants have for the sake of clarity amended claims 26 and 27 to remove the term "preferably." Additionally, claims 26 and 30 have been amended to remove the phrase "simultaneous, separate or sequential." No new matter has been introduced as a result of these amendments.

Claim Rejections – 35 U.S.C. § 103

Claims 1-2, 4, 7-21, 30, 35-38, 44-45 and 53-56 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Cramer, EP 0780127 (hereinafter "*Cramer*").

Claims 22 and 26-27 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over *Cramer* in view of Modi, U.S. Patent No. 6,294,153 (hereinafter "*Modi*").

Claims 1-2 and 6 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over *Cramer* in view of Fassberg, et al., U.S. Patent No. 6,416,743 (hereinafter "*Fassberg*").

Claims 1, 25, and 28-29 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over *Cramer* in view of Alfonso, et al., U.S. Patent No. 6,017,963 (hereinafter "*Alfonso*").

Claims 25, 28, and 29 are currently canceled. Accordingly, the pending claims stand or fall on the above-recited application of the primary reference, *Cramer*, alone or in combination with the secondary references, *Modi* or *Alfonso*, to independent claims 1, 26, 55, and 56. Applicants respectfully submit the pending claims are patentable in view of the cited references and provide herewith objective evidence of nonobviousness in that the claimed species directed to a pharmaceutical formulation comprising azelastine and fluticasone displays unexpectedly beneficial properties, is commercially successful, and fills a long felt but unsolved need.

The Legal Standard for Obviousness

The MPEP provides that "establishing a *prima facie* case of obviousness" requires, "the clear articulation of the reason(s) why the claimed invention would have been obvious." *See* MPEP § 2142. The MPEP also acknowledges that "[t]he Supreme Court in *KSR* noted that the analysis supporting a rejection under 35 U.S.C. 103 should be made explicit." *See* MPEP § 2143.

Moreover, in *KSR Int'l Co. v. Teleflex, Inc.*, the United States Supreme Court explained that, "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art," but, additionally whether "the claim extends to what is obvious." *See KSR Int'l Co. v. Teleflex, Inc.*, 82 USPQ2d 1385, 1397 (2007). Expounding on its edict, the Supreme Court went on to opine that an obviousness determination is based upon a "proper application of *Graham*," including consideration of "secondary factors" that may weigh against an obviousness determination. *See KSR Int'l Co. v. Teleflex, Inc.*, 82 USPQ2d at 1399 (citing *Graham v. John Deere Co. of Kansas City, et al.*, 383 U.S. 1, 148 USPQ 459 (1966)). The Office Action states:

> [t]he factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art. indicating obviousness or nonobviousness.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

A. *Cramer* does not fairly suggest the elected species

In ascertaining the difference in the prior art and the pending claims, the Office Action dated January 23, 2009 (hereinafter *OA 01232009*) acknowledges "Cramer does not exemplify a composition comprising azelastine and fluticasone." *See OA 01232009* at 12. As such, the Office Action retreats to a "rationale-based" obviousness rejection based on the conclusion that:

> one of ordinary skill in the art would have been motivated to make a composition comprising azelastine and fluticasone because Cramer suggests that the combination of a gluccocortoid (i.e. fluticasone) and antihistamine (i.e. azelastine) provide improved relief of symptoms associated with seasonal or perennial allergic rhinoconjunctivitis.

See OA 01232009 at 12.

The Office Action then supports its "rationale-based" rejection by stating, "the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made **because the prior art is fairly suggestive of the claimed invention**." *See OA 01232009* at 13 (emphasis added). The present Office Action maintains this position asserting that "[i]t is well within the means for one of ordinary skill in the art to try the instant combination as there are a small number of actives to **choose** from." *See* Office Action at 15, emphasis added. The Office Action's remark suggests a reliance on the KSR ruling and is asserting that it would have been "obvious to try" the instantly claimed combination.

Applicants submit the Office Action's rationale fails as it improperly applies the "obvious to try" standard. In *Kubin*, the Federal Circuit recognized that KSR "resurrects this court's own wisdom in *In re* O'Farrell" and addressed the question of "when is an invention that was obvious to try nevertheless nonobvious?" *In re Kubin*, 561 F.3d 1351, 1359(Fed. Cir. 2009) (citing *In re O'Farrell*, 853 F. 2d 894, 903(Fed. Cir. 1988)). In *Kubin*, the court described a class of cases where 'obvious to try' was erroneously equated with obviousness under § 103 as

> what would have been 'obvious to try' would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art either gave no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful.

See id, emphasis added. The court in *Kubin* made clear that "where a defendant merely throws metaphorical darts at a board filled with combinatorial prior art possibilities, courts should not succumb to hindsight claims of obviousness." *See id.*

Applicants contend that *Cramer* does not provide any guidance as to which of the number of combinations disclosed were critical or likely to be successful in producing the beneficial results disclosed by Applicants. Absent such guidance, the only disclosure of record regarding the beneficial properties associated with the combination of azelastine and fluticasone is that of the instant application. Such hindsight reconstruction of the instant invention traverses the mandate of MPEP § 2142 that "hindsight must be avoided and the legal conclusion must be reached on the basis of the facts gleaned from the prior art." Based on the foregoing, Applicants respectfully submit that the Office Action does not present a *prima facie* case of obviousness with regard to the instant claims.

B. Secondary considerations indicate that the combination of azelastine and fluticasone is nonobviousness

Assuming, without conceding, that the Office Action's "rationale and motivation" discussion is sufficient, nevertheless, the Office Action's suggestion of a *prima facie* case of obviousness must fail because the unaddressed "secondary considerations" described below render the instant claims nonobvious. *See KSR Int'l Co. v. Teleflex, Inc.*, 82 USPQ2d at 1399. Applicants provide herewith a Rule 1.132 declaration of inventor Geena Malhotra and the accompanying Exhibits A-D setting forth evidence of the following secondary considerations of nonobviousness.

Exhibit A has been amended

Applicants draw the Examiner's attention to Exhibit A submitted herewith. Applicants present in Exhibit A values that are amended (as shown in redline) from those presented in the Exhibit A filed in response to Office Action dated July 23, 2009. The amended values represent clarifications and the remedying of typographical errors in the previously submitted data. These corrections/amendments do not have any impact on the arguments previously submitted during the prosecution of the application.

1. The combination of azelastine and fluticasone displays unexpected, beneficial results

A showing of unexpected results may rebut a *prima facie* case of obviousness, and is particularly applicable in the inherently unpredictable chemical arts where minor changes may yield substantially different results. *See e.g., In re Soni*, 34 USPQ2d 1684, 1687 (Fed. Cir. 1995). Exhibit A of the declaration demonstrates that the claimed pharmaceutical formulation comprising azelastine and fluticasone has unexpected and beneficial stability. As noted in paragraph 2 of the declaration:

> The results in Table II show that the individual active materials (e.g., azelastine.HCl, budesonide, and fluticasone propionate) have good stability, in that the impurity levels are fairly constant in all the tests. The results in Table II also show that the combination of azelastine and budesonide are relatively unstable, with varying, and high amounts of impurities developing during the tests. Surprisingly, the results for azelastine and fluticasone show good stability throughout the tests, as the amount of impurity remains constant and at a low level.

These tests demonstrate that there is a clear unexpected advantage in product stability in formulating azelastine with fluticasone rather than with other steroids such as budesonide. Improved product stability is extremely important in pharmaceutical compositions as is understood by those skilled in the art.

Furthermore, Exhibits B1 and B3 of the declaration demonstrate that a pharmaceutical formulation comprising azelastine and fluticasone has unexpected and beneficial efficacy when administered to patients. Specifically, Exhibit B1 notes that the use of DUONASE (a commercial pharmaceutical formulation comprising azelastine and fluticasone) "is very effective when compared [to] the available other nasal sprays." Likewise, Exhibit B3 notes (with emphasis added):

> DUONASE Nasal Spray is very very effective in all types of allergic rhinitis. Especially in "Seasonal allergic rhinitis", Fluticasone alone or azelastine alone also has been tried. But single drug was not effective as compared with the combination of both i.e. "DUONASE Nasal Spray".

Likewise, the remainder of the doctor statements in Exhibit B extol the therapeutic benefits of the claimed pharmaceutical formulation comprising azelastine and fluticasone. Such recognition by skilled artisans of the merits of the invention is further evidence of nonobviousness. *See Akzo N.V. v. United States Int'l Trade Comm'n*, 1 USPQ2d 1241, 1247 (Fed. Cir. 1986). These doctor statements demonstrate a clear, unexpected advantage in treatment efficacy, namely that the combination of azelastine and fluticasone provides a synergistic benefit in efficacy over azelastine alone or fluticasone alone.

As set forth above, the declaration provides strong evidence that the claimed pharmaceutical formulation comprising azelastine and fluticasone has unexpected and beneficial stability, and that upon administration to a patient, unexpected and beneficial enhanced efficacy is observed. Accordingly, the claimed pharmaceutical formulation comprising azelastine and fluticasone is nonobvious in view of these unexpected results.

Response to alleged deficiencies of 1.132 Declaration

The Office Action asserts four alleged deficiencies of the previously submitted inventor declaration. *See* Office Action at 15 and 16. Without conceding that such deficiencies are present in the aforementioned declaration, Applicants will proceed to address these allegations in an effort to substantively advance prosecution of the instant application.

The Office Action first alleges there is no description of the testing method, assay utilized or how the impurity level was calculated. *See id.* Applicants provide herewith Exhibit D which describes the HPLC methodologies utilized for obtaining the stability data reported in Exhibit A. Particularly, Exhibit D provides conditions for HPLC analysis of the compositions discussed in Exhibit A and spectrophotometric detection of the indicated materials. Secondly, Exhibit D also identifies the nature of the impurities monitored for each composition. Applicants respectfully submit Exhibit D remedies the alleged deficiencies described in the Office Action with regard to Exhibit A and request reconsideration of the experimental showings provided in Exhibit A which support the nonobviousness of the claimed subject matter.

Thirdly, the Office Action's asserts that "Applicant did not test against the closest prior art examples described in *Cramer* (see Example 3). Example 3 in *Cramer* discloses a composition comprising azelastine and triamcinolone." *See* Office Action at 16. However, Applicants note that *Cramer* specifically treats fluticasone and budesonide as alternatives. *See Cramer*, claim 3. In

view of the teachings of the Office Action's cited reference, *Cramer*, the ordinarily skilled artisan would consider the appropriate comparatives to be that of azelastine and fluticasone to azelastine and budesonide. Applicants respectfully submit that such comparatives which are made in the aforementioned declaration are both appropriate and convincing as to the beneficial features associated with the azelastine/fluticasone composition.

Fourth and finally, Applicants note the Office Action's remarks with regard to the compositions described in Exhibit A that contain fluticasone also contain phenyl ethyl alcohol, a preservative/antibacterial. Particularly, the Office Action contends

> It is neither unexpected nor surprising that a composition comprising an additional preservative would be capable of keeping impurity levels lower and increasing shelf life when compared to a composition that does not contain the preservative or a lesser amount of the preservative.

See Office Action at 16-17. Applicants submit that the Office Action's analysis of the experimental results presented in Exhibit A is incomplete. Attention is respectfully directed to Exhibit A, Table 2 wherein the comparative stability of azelastine, budesonide, and fluticasone is presented. Budesonide in the absence of phenyl ethyl alcohol displays a total impurity level ranging from 0.25 to 0.49 over the course of the stability study. Fluticasone *in the presence of phenyl ethyl alcohol* over the course of the stability study displayed a range in the impurity level of from 0.46 to 0.53. Azelastine in the absence of phenyl ethyl alcohol shows a range in the impurity level over the course of the stability study of from 0.03 to 0.18. The ordinarily skilled artisan would surmise based on the information presented in Exhibit A that azelastine, fluticasone and budesonide independently exhibited similar stabilities over the course of the stability study. The presence of phenyl ethyl alcohol did not serve to distinguish the stability of the fluticasone sample from that of the azelastine or budesonide samples. To the contrary, budesonide samples and

azelastine samples in the absence of phenyl ethyl alcohol have a stability similar to that of fluticasone samples which contain phenyl ethyl alcohol. Applicants submit that the presence of phenyl ethyl alcohol in the azelastine and fluticasone composition cannot account for the observed dramatic increase in stability of this composition when compared to the azelastine and budesonide composition.

Further, Applicants provide herewith excerpts from the Handbook of Microbiological Quality Control and an article entitled "Preservatives in Ophthalmic Formulations." According to both these references, preservatives act on micro-organisms and help in protecting the formulation from them. None of these references mention the effect of preservatives on the chemical stability of the actives or drug. Thus, it is simply the assumption of the Office Action that the preservative *may* have an effect on the chemical stability of the actives.

The Office Action also makes statements that addition of a preservative prevents the decomposition of a substance or inhibits the multiplication of organisms which also causes decomposition. *See* Office Action at 15. The Office Action then refers the Applicants to two general references regarding the use of preservatives and cites a passage in *Cramer* regarding preservatives. However, the Office Action fails to establish that the microorganisms whose growth are inhibited by phenyl ethyl alcohol inherently impact the stability of azelastine and/or fluticasone but rather that such organisms *may* impact the stability of these materials. The fact that a certain result or characteristic **may** occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art) (emphasis added); *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). "To

establish inherency, the extrinsic evidence must make clear that the missing descriptive matter is **necessarily present** in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' " *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (emphasis added). As the Office Action has failed to establish that microorganisms inhibited by the presence of phenyl ethyl alcohol *necessarily* affect the stability of azelastine and/or fluticasone, Applicants respectfully assert that the submitted experimental showings would lead one of ordinary skill in the art to conclude the azelastine and fluticasone composition displays an unexpectedly beneficial stability when compared to the azelastine and budesonide composition. *See Inventor Declaration at 6.*

2. The combination of azelastine and fluticasone is commercially successful

Commercial success is a strong factor favoring nonobviousness. *See e.g., Akzo N.V.* at 1246. As noted in paragraph 4 of the declaration, a pharmaceutical formulation comprising azelastine and fluticasone is commercially available where approved as DUONASE nasal spray. The doctor statements set forth in Exhibit B provide further evidence of the commercial success of DUONASE nasal spray. Furthermore, as noted in paragraph 8 of the declaration the present application claiming a pharmaceutical formulation comprising azelastine and fluticasonse is licensed to Meda Pharmaceuticals, which specializes in respiratory, allergy, and cough-cold products. Given its expertise and knowledge in the field of treatment, the willingness of Meda Pharmaceuticals to license the pending application is further evidence of the commercial success of the claimed pharmaceutical formulation comprising azelastine and fluticasone. Accordingly, the claimed pharmaceutical formulation comprising azelastine and fluticasone is nonobvious in view of its commercial success.

3. The combination of azelastine and fluticasone fills a long-felt need

As set forth in *Graham*, the existence of a long-felt and unsolved need in the art is further evidence of nonobviousness. Applicants note that *Cramer* was published on June 25, 1997, which was over 10 years ago. Nonetheless, as noted in paragraph 7 of the declaration, inventor Geena Malhotra is unaware of another commercially available pharmaceutical formulation comprising an antihistamine and a steroid. Likewise, the doctor statement of Exhibit B4 notes that:

> I have been using nasal sprays from the year 1993, ever since I joined my present institution. I have used Beclomethasone, Budesonide, Azelastine, Fluticasone, Mometasone, with oral antihistamines down the line till date.
>
> The present combination spray of a weak (non sedating component) Azelastine and fluticasone (steroid component) is complete by itself in my patients of chronic simple rhinitis following nasal + sinus polyposis surgery and those unwilling for surgery or unfit for surgery.

Such "[f]irsthand practical knowledge of unsolved needs in the art, by an expert, is evidence of the state of the art." *See In re Piasecki*, 223 USPQ 785, 789 (Fed. Cir. 1984). Applicants respectfully submit that the evidence establishes a long-felt need dating back to 1993 that continued unsolved even after the subsequent publication of *Cramer* in 1997. Applicants further submit that the lack of another commercially available pharmaceutical formulation comprising an antihistamine and a steroid further evidences a long-felt need and the failure of others to address the need prior to the present invention. Accordingly, the claimed pharmaceutical formulation comprising azelastine and fluticasone is nonobvious given that it meets the long-felt need outlined above.

4. The secondary considerations require a finding of nonobviousness

As set forth above, the claimed pharmaceutical formulation comprising azelastine and fluticasone displays unexpected, beneficial results; is commercially successful; and fills a long-felt need in the art. Accordingly, the totality of the secondary considerations requires a finding that the pending claims are not obvious, and therefore patentable, in view of the prior art of record.

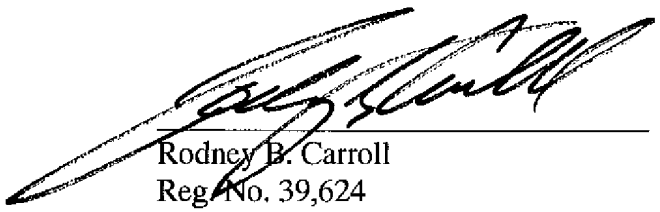
CONCLUSION

Consideration of the foregoing amendments and remarks, reconsideration of the application, and withdrawal of the rejections are respectfully requested by Applicants. No new matter is introduced by way of the amendment. It is believed that each ground of rejection raised in the Final Office Action dated April 28, 2010 has been fully addressed. If any fee is due as a result of the filing of this paper, please appropriately charge such fee to Deposit Account Number 50-1515 of Conley Rose, P.C., Texas. If a petition for extension of time is necessary in order for this paper to be deemed timely filed, please consider this a petition therefore.

If a telephone conference would facilitate the resolution of any issue or expedite the prosecution of the application, the Examiner is invited to telephone the undersigned at the telephone number given below.

Respectfully submitted,
CONLEY ROSE, P.C.

Date: 9-24-10



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Handbook of Microbiological Quality Control

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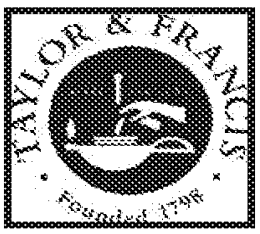

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10

Antimicrobial Preservative Efficacy Testing

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10.1 Introduction

A wide variety of products need to be protected from attack by micro-organisms during their period of use. This is both to protect the user from the dangers of infection and to prevent spoilage and deterioration of the product. In the case of medicines, foods and cosmetics, the safety of the user is the main priority, but maintenance of product quality and appearance and suitability of the product for its intended purpose are also important.

Preservatives are intended to protect the product from spoilage due to organisms introduced by the user and those which unavoidably arise during the manufacturing process; preservatives should never be used to counter poor manufacturing procedures or poor-quality ingredients. Clearly, sterile products in single dose units do not require preservation, neither do non-sterile single dose units such as tablets and capsules which are unlikely to sustain microbial survival provided that they are contained within suitable packaging. The need for a preservative system, therefore, most commonly arises if the product is to be subject to microbial challenge during repeated use. Some products are self-preserving, either because the active ingredients are inhibitory, the pH is inimical to growth, or because they contain high concentrations of sugar or other solutes which act as osmotic preservatives. These types of formulations are rare in the pharmaceutical arena, and the majority of multi-dose water-containing medicines incorporate chemical preservatives to prevent microbial spoilage.

The term preservative describes the *function* of a chemical agent in protecting a product from degradation or change which might arise if micro-organisms were to gain access and grow in it. However, this can be misleading since it might be thought that preservatives merely maintain the *status quo* (prevent micro-organisms growing, but not necessarily kill them), and as a result it is not uncommon to encounter the phrase *preservative levels of biocide* implying low concentrations of chemical agents which have only a bacteriostatic effect. In the majority of cases, however, the concentrations of preservatives used in product formulations are designed to give a rapid kill of any invading micro-organisms. Increasingly, preservatives are used in

PRESERVATIVES IN OPHTHALMIC FORMULATIONS: AN OVERVIEW

GENERALIDADES DE LOS CONSERVANTES EN LAS FORMULACIONES OFTÁLMICAS

HERRERO VANRELL R[1]

In certain ocular pathologies, ophthalmic formulations need to be chronically administered in order to guarantee their efficacy. Typical examples of such pathologies are dry eye and glaucoma. Nevertheless, although preservatives have been frequently used in eye drops, its frequent use has been associated with alterations in the precorneal film, while in patients suffering from dry eye they tend to aggravate the already existing problem. On the other hand, in glaucoma patients the prolonged use of eye drops with preservatives has been associated with changes in the ocular surface accompanied by inflammation. In fact, conjunctival byopsies in patients suffering from glaucoma have revealed an increased number of immune cells and fiberblasts (1,2).

Thanks to the experience garnered so far, we can say that the successive administration of formulations with preservatives has a toxic effect in the ocular surface and in particular in those patients whose surface is compromised. However, as stated by the Real Farmacopea Española (RFE), the use of preservatives is mandatory in the case of multidose formulations, since bacterial contamination takes place when handling containers twice a day for two weeks. As quoted by the RFE (3): *Aqueous formulations in multidose containers shall include the appropriate antimicrobial preservative at adequate concentrations in order to prevent tampering of preparations during the time of use, except in those instances when preparations feature sufficient antimicrobial properties.*

A wide number of preservatives is used in the formulation of eye drops, among them benzalkonium chloride, benzethonium chloride and cetyl pyridinium chloride, benzyl bromide, EDTA, phenylmercury nitrate, phenylmercury acetate, thimerosal, merthiolate, acetate and phenylmercury borate, polymyxin B sulphate, chlorhexidine, methyl and propyl parabens, phenylethyl alcohol, quaternary ammonium chloride, sodium benzoate, sodium propionate and sorbic acid.

Progress in the treatment of dry eye has been linked to the emergence of new preservatives in the market based on stabilized chloride and oxygen compounds (Purite®) as well as sodium perborate (4). These agents have raised enormous interest since they were effective and apparently did not entail epithelial damage as other conventional drugs did. In any case, one of the most significant advances in the treatment of dry eye was the development of preservative-free artificial tears in monodose containers or else the inclusion of a sterilizing filter in multidose containers (Sistema Abak®).

The action mechanism of preservatives may be divided into two main categories: surfactants and oxidants (1,2).

Surfactants act upon microorganisms altering the cellular membrane and resulting in the lysis of the cytoplasm content. Cells in mammals cannot neutralize chemical preservatives, and thus preservatives become part of the cell and results in toxic effects. The classical example for this type of agents is benzalkonium chloride.

Oxidizing preservatives are usually smaller molecules interfering with cell functions. They may destabilize membranes, although to a lesser extent than chemical agents may. They are less toxic for mammal cells, which are equipped with enzymes capable of catalyzing the decomposition of hydrogen peroxide as long as preservatives are found in

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low concentrations. Stabilized chlorine and oxygen compounds and sodium perborate are some examples of oxidizing preservatives.

Taking into account their impact on the corneal epithelium, it is clear that preservatives should not be used when there is some kind of trauma or in patients who have undergone a surgical procedure, since there is a risk of causing irritation in the anterior chamber. We need to take into consideration the fact that these agents are exclusively devoted to preventing the potential contamination of solutions by microorganisms during the use of this medication and should not to be included in formulations for intraocular use.

Another relevant aspect to take into account is that the intermittent use of formulations with preservatives needs not to be theoretically linked to adverse side effects. However, the use of several eye drops at the same time increases exposure to preservatives, since the concentration to which the ocular surface is exposed increases together with the number of applications. Furthermore, repeated doses may result in the accumulation of preservatives.

Obviously, the use of preservatives in ophthalmic formulations is necessary and cannot be avoided. Nevertheless, we should determine which preservatives induce less toxicity in epithelial and conjunctival cells. Cellular lines and cellular feasibility trials are efficient tools to bring about these studies.

REFERENCES

1. *Noecker R. Ophthalmic preservatives: considerations for long-term use in patients with dry eye or glaucoma. Rev Ophthalmol 2001; 8: 73-79.*
2. *Noecker R. Effects of common ophthalmic preservatives on ocular health. Adv Ther 2001; 18: 205-215.*
3. *Real Farmacopea Española. III ed. Madrid: Ministerio de Sanidad y Consumo; 2007.*
4. *Bartlett JD. Ophthalmic Drug Facts. St. Louis: Wolters Kluwer Health Inc; 2006.*
5. *United States Pharmacopeia Convention. USP 30. NF 25. The United States Pharmacopeial. The National Formulary. Rockville: 2007.*

Development of fluticasone propionate and comparison with other inhaled corticosteroids

Malcolm Johnson, PhD *Middlesex, United Kingdom*

Fluticasone propionate (FP) is a trifluorinated glucocorticoid based on the androstane nucleus. It was selected for development from structure-activity relationships (topical anti-inflammatory, cutaneous vasoconstriction, and hypothalamic-pituitary-adrenal axis suppression) of a series of 17β-carbothioates. FP is 3-, 300-, and 1000-fold more lipophilic than beclomethasone dipropionate, budesonide, and triamcinolone acetonide, respectively. FP has an absolute affinity (K_D) for the glucocorticoid receptor of 0.5 nmol/L and a relative receptor affinity 1.5-fold higher than beclomethasone-17-monopropionate (17-BMP) and mometasone furoate, 3-fold higher than budesonide, and 20-fold higher than flunisolide and triamcinolone acetonide. The rate of association of FP with the receptor is faster and the rate of dissociation slower than other corticosteroids. The resulting half-life of the FP active steroid-receptor complex is >10 hours, compared with approximately 5, 7.5, and 4 hours for budesonide, 17-BMP, and triamcinolone acetonide, respectively. FP has high selectivity for the glucocorticoid receptor, with little or no activity at other steroid receptors. FP is more potent than beclomethasone dipropionate, budesonide, triamcinolone acetonide, and mometasone furoate in inhibiting human T-cell migration and proliferation, inhibiting CD4+ T-cell cytokine and basophil histamine release, attenuating adhesion molecule expression, stimulating inflammatory cell apoptosis, and inducing cellular antiprotease release. In asthma patients, FP decreases the number of CD3+, CD4+, CD8+, and CD25+ T cells, mast cells, and eosinophils in bronchial biopsies, in addition to suppressing CD1a-dendritic and IgE+ cells and HLA-DR. FP, therefore, has a good pharmacologic profile for a topical steroid with increased intrinsic glucocorticoid potency and potent anti-inflammatory activity.
(J Allergy Clin Immunol 1998;101:S434-9.)

Key words: *Fluticasone propionate, inhaled corticosteroids, structure-activity relationships, asthma*

To exert anti-inflammatory activity, a corticosteroid molecule must penetrate the cellular membrane and demonstrate affinity for the steroid binding site on the glucocorticoid receptor (GR), leading to activation of the receptor.[1] Dimerization of the active steroid-receptor complex occurs, and this can then enter the nucleus, bind to glucocorticoid-responsive elements on a target gene, influence gene transcription, and either inhibit proinflammatory or potentiate endogenous anti-inflammatory mechanisms. Alternatively, a direct interaction of the GR complex with transcription factors may also be an important determinant of steroid action and a key mechanism by which glucocorticoids exert some anti-inflammatory activity.[1]

Abbreviations used

BDP:	Beclomethasone dipropionate
17-BMP:	Beclomethasone-17-monopropionate
FP:	Fluticasone propionate
GR:	Glucocorticoid receptor
GRE:	Glucocorticoid-responsive element
RBA:	Relative receptor binding affinity

The early development of corticosteroids based on the structure of cortisol focused on increasing topical potency and improving glucocorticoid selectivity. The first structure-activity studies attempted to find compounds with greater anti-inflammatory activity. This was achieved either by the insertion of an additional double bond at the 1,2 position in the steroid nucleus; by the introduction of 6α-fluoro, 6α-methyl, or 9α-fluoro substituents; or by a combination of these changes (Fig. 1). Although anti-inflammatory potency was potentiated, mineralocorticoid activity was increased to an even greater extent.[2] This effect was counteracted by further substitutions with α-hydroxyl, α-methyl, or β-methyl at the 16 position, for example, in dexamethasone (Fig. 1). A novel finding was that an ester function at the 16α, 17α, or 21α hydroxyl group was preferred, and this gave rise to betamethasone 17-valerate, triamcinolone 16,17-acetonide, and beclomethasone-17,21-dipropionate.[2] These compounds have proved to be of value in the treatment of the inflammatory component of bronchial asthma and rhinitis and have shown little detectable systemic activity when delivered by the topical route. However, concern that long-term therapy may result in a wide range of unacceptable systemic side effects such as adrenal suppression, bone fracture, osteoporosis, and inhibition of growth in children highlighted the need for steroids with a better therapeutic index.

DEVELOPMENT OF FLUTICASONE PROPIONATE

The development of fluticasone propionate was an attempt to produce a potent corticosteroid that exhibited improved airway selectivity (Table I) compared with earlier compounds. Lipophilicity was identified as an important physicochemical property for increased uptake and retention in lung tissue, resulting in enhanced lung-systemic distribution and greater affinity for the

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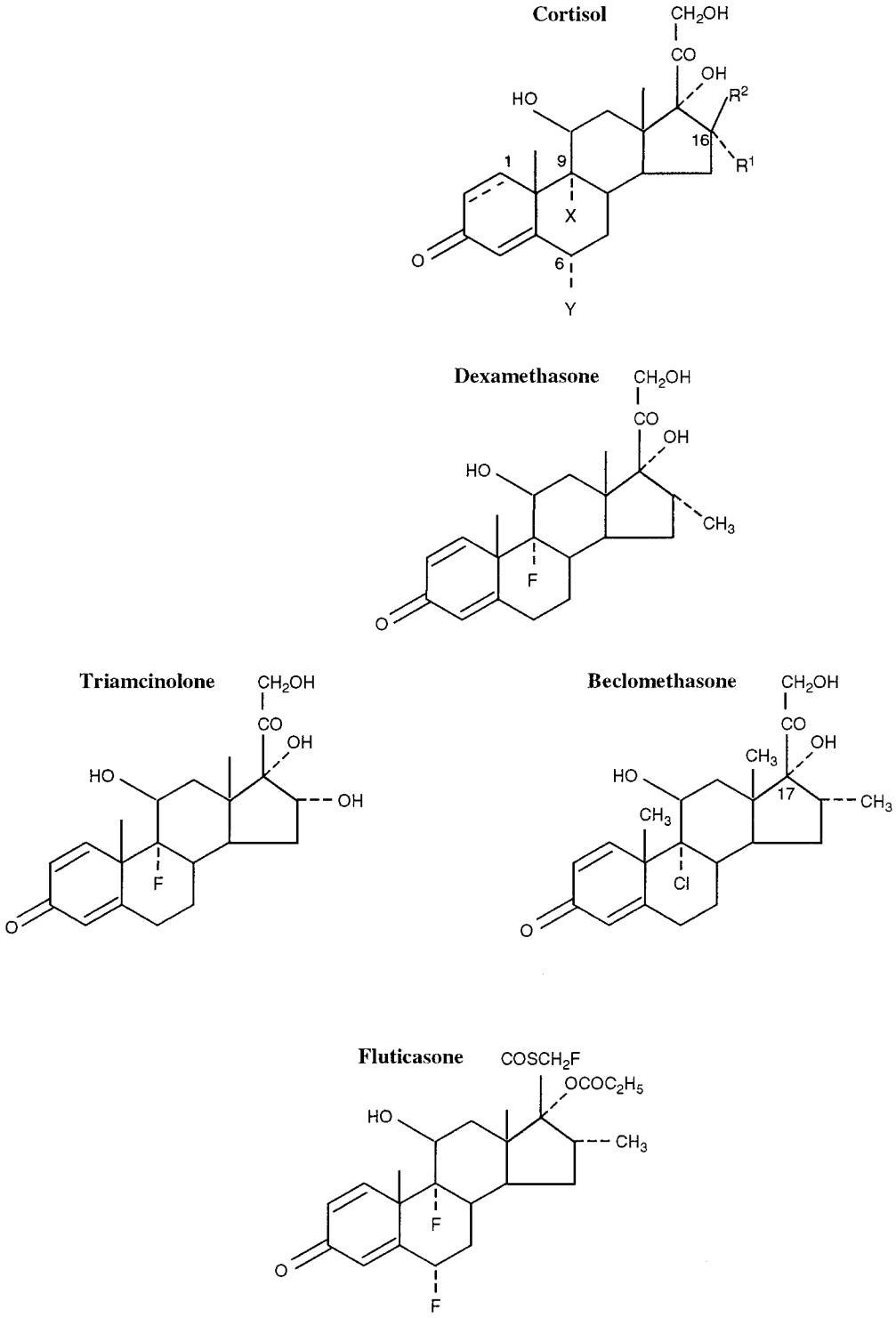

FIG. 1. Structural modifications of cortisol that produced the corticosteroids: dexamethasone, triamcinolone acetonide, beclomethasone dipropionate, and fluticasone propionate.

GR. The androstane nucleus, which is highly lipophilic, was therefore selected as the basis of the chemical program.[3] Topical activity was assessed by inhibition of croton oil–induced inflammation of the ear in a mouse model[4] and inhibitory activity at the hypothalamic-pituitary-adrenal (HPA) axis assessed by measuring reductions in circulating corticosteroids in response to ether stress.[5] The vasoconstriction/skin blanching assay[6]

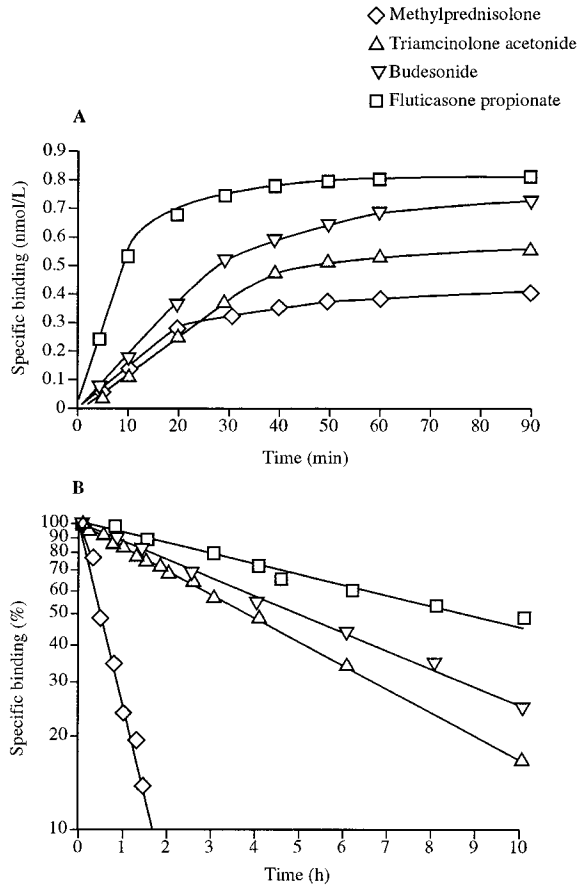

FIG. 2. Kinetics of **(A)** association and **(B)** dissociation of methylprednisolone, triamcinolone acetonide, budesonide, and fluticasone propionate with the glucocorticoid receptor in human lung tissue. Data from references 10 and 14.

was then used to confirm activity in human beings and to rank compounds in order of anti-inflammatory potency.

The androstane 17β-carboxylates, which lack the normal two-carbon side-chain of anti-inflammatory corticosteroids at the 17 position, were of particular interest.[3] The 17α-hydroxyl,17β-carboxylic acid was without activity in the vasoconstriction assay, with esterification being necessary for topical activity. Enzymatic hydrolysis of either ester function, which can occur in vivo, would therefore lead to inactive metabolites. The 17β-carboxylate series was superseded by the corresponding 17β-carbothioates.[3] Fluoromethyl analogues were, in general, more active than the corresponding chloromethyl compounds, with the 17-propionate being preferred over the acetate or butyrate; in addition, the presence of an α-CH_3 at position 16 reduced HPA axis–suppressing activity (Table II). The most active compound in the anti-inflammatory and vasoconstriction tests was the 6α,9α-difluoro, 17α-propionyl, 17β-carbothioate (fluticasone propionate), which was approximately 2-fold and 10-fold more potent than BDP and fluocinolone acetonide, respectively (Table II). Its low activity in inhibiting HPA axis function resulted from FP undergoing complete first-pass metabolism in the liver to the inactive 17β-carboxylic acid. X-ray crystallography has shown that the carbonyl of the 17β-substituent lies below the plane of the ring rather than above it, which is observed for other anti-inflammatory steroids.[7] This unusual shape, in which the carbothioate ester has increased accessibility, may explain why FP readily undergoes enzymatic hydrolysis. FP therefore has a high calculated therapeutic index (anti-inflammatory potency/HPA inhibitory potency) of 91, compared with 0.4 and 1.0 for BDP and fluocinolone acetonide, respectively.[8]

TABLE I. Criteria for improved airway selectivity of corticosteroids

Pharmacodynamics
• High glucocorticoid receptor affinity
• Optimal glucocorticoid receptor kinetics
• High intrinsic steroid potency/high topical anti-inflammatory activity
• High glucocorticoid receptor specificity
Pharmacokinetics
• Low oral bioavailability
• Increased uptake/retention in lung tissue
• Rapid systemic clearance
• Extrapulmonary metabolism to inactive metabolite(s)
• High lung:systemic distribution ratio

FP is 3 and 300 times more lipophilic than BDP and budesonide, respectively, and >1000-fold more lipophilic than either flunisolide or triamcinolone acetonide.[9] This degree of lipophilicity gives FP increased deposition in lung tissue and a slower release from the lung lipid compartment. In human lung fragments and nasal tissue in vitro, uptake and retention of corticosteroids is in the rank order FP > BDP > 17-BMP > budesonide > flunisolide > hydrocortisone.[10, 11] In patients with asthma, after inhalation of a 1 mg dose, FP exhibits a lung:systemic distribution ratio of 70 to 100,[12] compared with previous reports of 7 to 10 for budesonide.[13]

RECEPTOR PHARMACOLOGY

FP has a high affinity for the human lung GR (0.5 nmol/L),[14] which is 1.5-fold higher than 17-BMP and mometasone furoate, 3-fold higher than budesonide, and 10-fold higher than triamcinolone acetonide and flunisolide (Table III). Unlike budesonide, which is a racemic mixture of 22R and 22S enantiomers, FP does not have a chiral center and therefore the measured affinity represents the affinity of the molecule and not the contribution of the individual enantiomers. In contrast to 17-BMP, the metabolite of BDP that has a relative receptor binding affinity (RBA) 5-fold higher than the parent molecule, budesonide, with an RBA of 7.8, undergoes a marked reduction in activity when metabolized to either 6-hydroxy-budesonide (RBA = 0.06) or 16-α-hydroxy-prednisolone (RBA = 0.03). The

TABLE II. Structure-activity of halomethyl-androstane-17β-carbothioate analogues

Z	Y	X	R	16	Topical anti-inflammatory activity*	HPA suppression†	Cutaneous vasoconstriction‡
F	H	CI	C_2H_5	H	20	100	916
F	H	F	C_2H_5	H	63	149	1984
F	F	CI	C_2H_5	αCH_3	56	0.04	124
F§	F	F	C_2H_5	αCH_3	113	1.0	945
F	F	F	CH_3	αCH_3	76	2.9	392
F	F	F	C_3H_7	αCH_3	55	0.7	299
F	F	F	C_2H_5	βCH_3	197	>100	1048

Results are expressed relative to fluocinolone acetonide as standard (100). Data from Reference 3.
*Assessed with the croton oil ear assay in mice.[4]
†Assessed with the ether stress assay in rodents.[5]
‡Assessed with the skin blanching test in human volunteers.[6]
§Structure of fluticasone propionate.

TABLE III. Comparison of corticosteroid-glucocorticoid receptor affinity in human lung and potency in the cutaneous vasoconstriction test

Corticosteroid	Relative glucocorticoid receptor affinity*	Relative vasoconstrictor activity†
Fluocinolone acetonide	1.0	1.0
Beclomethasone-17-monopropionate	3.3	2.0
Triamcinolone acetonide	0.5	0.4
Flunisolide	0.45	0.5
Mometasone furoate	3.3	3.0
Budesonide	2.5	1.5
Fluticasone propionate	5.0	5.0

Activities are quoted relative to fluocinolone acetonide as standard (1.0).
*Data from Reference 14.
†Data from Reference 6.

TABLE IV. Corticosteroid-induced inhibition of human inflammatory cells

Corticosteroid	IC_{50} (nmol/L)			
	T-cell IL-5 release*	T-cell proliferation†	Basophil histamine release‡	Eosinophil apoptosis§
Beclomethasone dipropionate	7.7	10.0	1.0	138.7
Triamcinolone acetonide	9.8	1.0	20.0	23.8
Budesonide	1.7	0.2	0.6	8.5
Mometasone furoate	0.3	. . .	0.3	. . .
Fluticasone propionate	0.2	0.05	0.03	1.7

*Data from Reference 19.
†Data from Reference 18.
‡Data from Reference 20.
§Data from Reference 21.

17β-carboxylic acid metabolite of FP has negligible pharmacologic activity, with an RBA <0.01 at the GR.[9] The rate of association of steroid with the cytosolic GR is fastest for FP, followed by budesonide, triamcinolone acetonide, and methyl prednisolone (Fig. 2). In contrast, the rate of dissociation of FP from the receptor complex is slower than that of budesonide, triamcinolone acetonide, dexamethasone, and methyl prednisolone (Fig. 2). These differences in GR kinetics for FP result in differences in the stability of the steroid-receptor complex, which mediates the biologic and therapeutic activity of glucocorticoids.[1] The half-life of the steroid-receptor complex for FP is >10 hours, compared with approximately 3.5, 4.0, 5.0, and 7.5 hours for flunisolide, triamcinolone acetonide, budesonide, and 17-BMP, respectively.[9] FP is highly selective for the GR with <0.001 of the relative potency at human androgen, estrogen, and mineralocorticoid receptors.[15] The selectivity ratio of FP for the GR over the progestagen receptor is 1430, compared with 267 and 237 for 17-BMP and budesonide, respectively.

ANTI-INFLAMMATORY ACTIVITY

The steroid receptor profile of FP imparts a high topical anti-inflammatory activity. The active FP-GR complex binds to the GRE on target genes (EC_{50} = 3 nmol/L) or interacts directly with activating protein-1 and/or nuclear factor-kB transcription factors (EC_{50} range 0.01 to 0.1 nmol/L) at significantly lower concentrations than either dexamethasone or budesonide.[16] This has a good correlation with the respective potency of FP in inhibiting GRE-dependent cytokine (IL-6, IL-8) synthesis (IC_{50} range 5 to 10 nmol/L) and non–GRE-dependent cytokines such as tumor necrosis factor-α (TNFα) and granulocyte-macrophage colony stimulating factor (IC_{50} range 0.01 to 0.1 nmol/L).

There is also a good correlation between the relative affinity of these corticosteroids for the GR and their relative potency in a number of intact inflammatory cell systems (Table IV). For example, FP is more potent than dexamethasone, BDP, and budesonide in inhibiting human T-cell migration[17] and proliferation,[18] with IC_{50}

values of 0.3, 5.9, 2.0, and 0.8 nmol/L. Similarly, anti–CD3/CD28-induced IL-5 and IL-4 secretion from CD4+ T cells is inhibited by corticosteroids, with a rank order of potency of FP > mometasone furoate > budesonide > BDP > triamcinolone acetonide.[19] FP inhibits anti–IgE-stimulated histamine release from human basophils with an IC_{50} of 0.03 nmol/L, compared with 0.3, 0.6, 1, and 20 nmol/L for mometasone furoate, budesonide, BDP, and triamcinolone acetonide, respectively.[20] Corticosteroids, in the presence of IL-5, induce concentration-dependent apoptosis of eosinophils, with FP (EC_{50} = 1.7 nmol/L) being 5 times more potent than budesonide and approximately 10 times more potent than triamcinolone acetonide and flunisolide.[21] FP is also potent in inhibiting cytokine-induced adhesion molecule expression. At 1 nmol/L, FP inhibits TNFα-stimulated E-selectin in human endothelial cells,[22] whereas 8-fold higher concentrations of budesonide are required for the same effect. At a concentration of 100 nmol/L, FP is more effective than budesonide or triamcinolone acetonide in inhibiting intracellular adhesion molecule-1 expression in airway epithelial cells.[23] Finally, Abbin ante-Nissen et al.[24] have shown that corticosteroids induce the synthesis of the antiprotease, secretory leukocyte protease inhibitor (SLPI), in human airway epithelial cells. FP is the most potent steroid in inducing SLPI, with an EC_{50} of 0.1 nmol/L compared with 1, 5, and 2 nmol/L for triamcinolone acetonide, methylprednisolone, and dexamethasone, respectively.

The rank order of affinity of corticosteroids at the GR and their anti-inflammatory potency in vivo are similar. In the McKenzie test, in which the cutaneous vasoconstrictor and skin blanching response is used to rank anti-inflammatory potency of topical corticosteroids,[6] FP is 1.5-, 2.5-, and 3-fold more potent than 17-BMP, mometasone furoate, and budesonide, respectively, and 10-fold more potent than triamcinolone acetonide and flunisolide (Table III). This is in agreement with Dahlberg et al.,[25] who had previously reported that the RBA predicts relative potency for inhibition of edema.

CLINICAL STUDIES

In patients with asthma, FP treatment (1 mg twice daily for 2 months) significantly reduced the numbers of mast cells (by 80.2%), eosinophils (by 93.6%), and T cells (CD3, CD4, CD8, CD25; mean reduction of 86.5%) in bronchial biopsy specimens.[26] Similarly, the presence of dendritic (CD1a), IgE+, and HLA-DR+ cells in the lamina propria was decreased after FP 1 mg daily for 3 months,[27] suggesting attenuation of antigen recognition, processing, and presentation. Finally, FP (500 μg twice daily for 8 weeks) results in a marked decrease in the bronchoalveolar lavage levels of metalloprotease and an increase in the concentration of the endogenous tissue inhibitor of metalloproteases (TIMPS),[28] both of which have been implicated in matrix protein deposition and basement membrane thickening. FP, therefore, has good activity against the chronic inflammatory component of bronchial asthma and may attenuate the degree of airway remodeling.

The development of FP has resulted in a corticosteroid molecule with increased intrinsic glucocorticoid potency and potent anti-inflammatory activity, coupled with improved airway selectivity.[29] FP is of considerable clinical importance in the treatment of asthma and rhinitis.

REFERENCES

1. Barnes PJ, Adcock IM. Anti-inflammatory actions of steroids: molecular mechanism. Trends Pharmacol Sci 1993;14:436-41.
2. Phillipps GH. Locally active corticosteroids: structure-activity relationships. In: Wilson L, Markes R, editor. Mechanisms of topical corticosteroid activity. London: Churchill Livingstone; 1976. p. 1-18.
3. Phillipps GH, Bailey EJ, Bain BM, Borella RA, Buckton JB, Clark JC, et al. Synthesis and structure-activity relationships in a series of anti-inflammatory corticosteroid analogues, halomethyl androstane-17β-carbothiates and 17β-carboselenoates. J Med Chem 1994;37: 3717-29.
4. Tonelli G, Thibault L, Ringler I. A bio-assay for the concomitant assessment of the antiphlogistic and thymolytic activities of topically applied corticoids. Endocrinology 1965;77:625-34.
5. Zenker N, Bernstein DE. The estimation of small amounts of corticosterone in rat plasma. J Biol Chem 1958;231:695-701.
6. McKenzie AW. Percutaneous absorption of steroids. Arch Dermatol 1962;86:611-4.
7. Weeks CM, Duax WL, Wolff ME. A comparison of the molecular structures of six corticosteroids. J Am Chem Soc 1973;95:2865-8.
8. Phillipps GH. Structure-activity relationships of topically active steroids: the selection of fluticasone propionate. Respir Med 1990; 84(suppl):19-23.
9. Johnson M. Pharmacodynamics and pharmacokinetics of inhaled glucocorticoids. J Allergy Clin Immunol 1996;97:169-76.
10. Högger P, Rawert L, Rohdewald P. Dissolution tissue binding and kinetics of receptor binding of inhaled glucocorticoids. Eur Respir J 1993;6:584s.
11. Högger P, Bonsmann U, Rohdewald P. Efflux of glucocorticoids from human lung tissue to human plasma in vitro. Eur Respir J 1994;7:382s.
12. Högger P, Esmailpour N, Rabe K, Heitmann U, Nakashima N, Rhodewald P. Distribution of inhaled fluticasone propionate between lung tissue and blood plasma in vivo. Eur Respir J 1995;8: 303S.
13. Van den Bosch JMM, Westermann CJJ, Aumann J, Edsbacker S, Tönneffon M, Selrous O. Relationship between lung tissue and blood plasma concentrations of inhaled budesonide. Biopharm Drug Dispos 1993;14:455-9.
14. Högger P, Rohdewald P. Binding kinetics of fluticasone propionate to the human glucocorticoid receptor. Steroids 1994;59:597-602.
15. Harding SM. The human pharmacology of fluticasone propionate. Respir Med 1990;84(suppl):25-9.
16. Adcock IM, Barnes PJ. Ligand-induced differentiation of glucocorticoid receptor transrepression and transactivation. In: Acute and chronic inflammation in the respiratory tract, International Respiratory Forum. Reading UK: Colwood Publications; 1996. p. 25.
17. Cruikshank W, Masterson J, Center D. Effects of fluticasone propionate on random and stimulated T cell migration. In: Acute and chronic inflammation in the respiratory tract, International Respiratory Forum. Reading UK: Colwood Publications; 1996. p. 19.
18. English AF, Neate MS, Quint DJ, Sareen M. Biological activities of some corticosteroids used in asthma. Am J Respir Crit Care Med 1994;149:A212.
19. Umland SP, Nahrebne MS, Razac S, Billah MM, Egan RW. Effects of mometasone furoate and other glucocorticoids on cytokine production from cultured peripheral blood CD4+ T cells. J Allergy Clin Immunol 1996;97:423.

20. Bickel CA, Stellato C, Schleimer RP. Comparison of the effect of inhaled glucocorticoids on human basophil histamine release and eosinophil viability. J Allergy Clin Immunol 1997;99:1632.
21. Kita H, Hagen JB, Gleich GJ. Fluticasone propionate is the most potent glucocorticoid in the induction of apoptosis of eosinophils. In: Acute and chronic inflammation in the respiratory tract, International Respiratory Forum. Reading UK: Colwood Publications; 1996. p. 17.
22. Johnson M. Anti-inflammatory properties of fluticasone propionate. Int Arch Allergy Immunol 1995;107:439-40.
23. Adler KB, Krunkosky TA, Fischer BM. Effects of tumour necrosis factor-alpha on airway epithelial cells in vitro. In: Acute and chronic inflammation in the respiratory tract, International Respiratory Forum. Reading UK: Colwood Publications; 1996. p. 18.
24. Abbinante-Nissen JM, Simpson LG, Leikauf GD. Corticosteroids increase secretory leukocyte protease inhibitor transcript levels in airway epithelial cells. Am J Respir Crit Care Med 1994;149:A869.
25. Dahlberg E, Thalén A, Brattsand R, Gustafsson JA, Johansson U, Römpke K. Correlation between chemical structure, receptor binding, and biological activity of some novel, highly active, 16α, 17α-acetyl-substituted glucocorticoids. Mol Pharmacol 1983;25: 70-8.
26. Booth H, Gardiner PV, Ward C, Walls A, Hendrick DJ, Walters EH. Effect of fluticasone on airway inflammation as assessed by bronchoalveolar lavage (BAL). Eur Respir J 1993;6(suppl 17): 584S.
27. Möller GM, Overbeek SE, van Helden-Meeuwsen CG, de Jong TAW, Mulder PG, Drexloge HA, et al. Glucocorticoid therapy is associated with modulation of dendritic cells in bronchial mucosa of atopic asthmatic subjects. In: Acute and chronic inflammation in the respiratory tract, International Respiratory Forum. Reading UK: Colwood Publications; 1996. p. 16.
28. Warner JA, Shute JK, Howarth PH. Effect of therapy on matrix metallo-proteinase (MMP) activity in the broncho-alveolar lavage (BAL) of patients with mild asthma. Am J Resp Crit Care Med In press.
29. Derendorf H, Hochhaus G, Möllermann H, Barth J, Krieg M, Jurn S, et al. Receptor-based pharmacokinetic-pharmacodynamic analysis of corticosteroids. J Clin Pharmacol 1993;33:115-23.